EXPEDITED PROCEDURE – EXAMINING GROUP 1623

S/N 09/458,862 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Allison Hubel Examiner: Elli Peselev
Serial No.: 09/458,862 Group Art Unit: 1623
Filed: December 10, 1999 Docket: 600.451US1
Title: COMPOSITIONS AND METHODS FOR CRYOPRESERVATON OF PERIPHERAL BLOOD LYMPHOCYTES

DECLARATION UNDER 37 C.F.R. 1.132

Sir:

I, Allison Hubel, Ph.D., declare and say as follows:

1. I am the inventor of subject matter claimed in the above-identified application. I make this Declaration in support of the patentability of the claims of the above-identified application.

2. In the Office Action dated April 16, 2001, the Examiner rejected claims 1-12, 14-24 and 31-36 under 35 U.S.C. § 102(b) as anticipated by, or alternatively under 35 U.S.C. § 103(a) as obvious over, the LAREX Material Data Safety Sheet or WO 97/35472, and rejected claims 1-12, 14-24 and 26 under 35 U.S.C. § 102(a) as anticipated by, or alternatively under 35 U.S.C. § 103(a) as obvious over, WO 97/35472.

3. The LAREX Material Safety Data Sheet relates that Cellsep™ powder contains at least 99% arabinogalactan (AG), and that AG is approved as a food additive by the FDA. The LAREX Technical Data Sheet also discloses that Cellsep™ powder is a medium for density gradient cell separation which provides superior resolution of a wide variety of cell types and cellular organelles, and that Cellsep™ isotonic solutions are available for lymphocytes and platelets.

4. WO 97/35472 generally discloses that AG-containing cryopreservation media may be employed with a variety of cell types including blood cells, although the only data provided in the WO 97/35478 specification is for seven lines of immortalized

mammalian cells. These seven lines were frozen in six different media, five of which contained AG: medium 3 had 50% AG, medium 4 had 20% AG and 10% DMSO, medium 6 had 15% AG and 20% serum, medium 2 had 10% AG and 20% DMSO, and medium 5 had 10% AG, 10% DMSO and 20% serum. Medium 1 had 10% DMSO and 20% serum (no AG). WO 97/35472 also describes a general method of freezing cells (page 8 and Example 2). WO 97/35472 concludes that AG "can be used to replace serum in a standard freezing medium, in a formulation with DMSO, for all cell types studied" and that freezing in 50% w/v AG was better or equivalent to the standard media for 5/7 cell types tested.

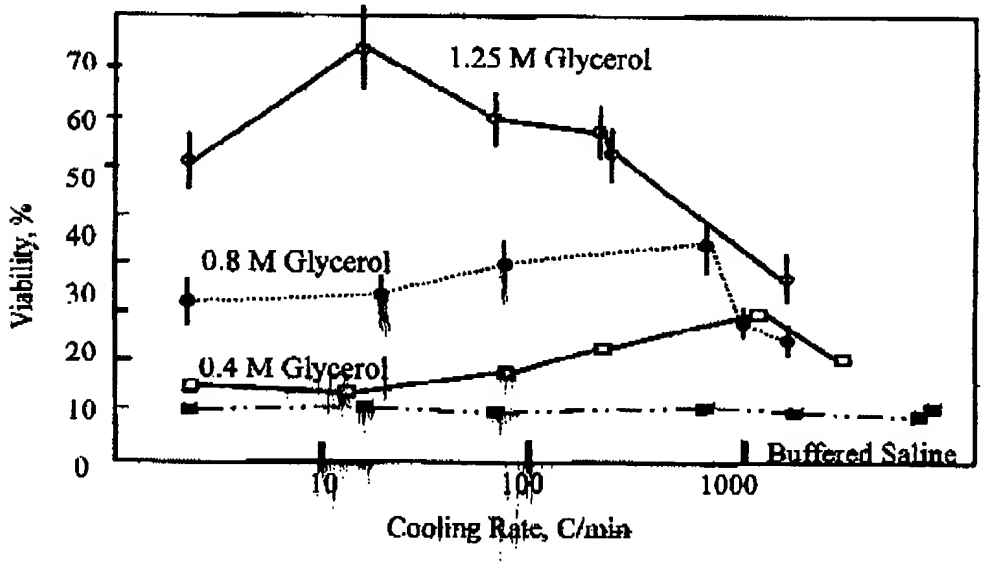
5. The pending claims are directed to compositions comprising AG, which are suitable for the cryopreservation of certain cell types, i.e., freshly isolated lymphocytes, stem cells or lymphocytes which have been modified *ex vivo*. A variety of interrelated factors influence the ability of cells to survive the stresses of freezing and thawing. Those factors include but are not limited to: (1) the composition of the cryoproservation solution; (2) the temperature history of the sample during cooling (e.g., cooling rate); and (3) the biological and biophysical characteristics of the cell/tissue being frozen. Some cell types, e.g., granulocytes, cannot be cryopreserved at all.

6. A cryopreservation solution typically includes a balanced salt solution such as tissue culture medium supplemented with one or more cryoprotective agents. These agents may include large molecular weight polymers or molecules, such as AG, that cannot penetrate the cell membrane by passive diffusion. These cryoprotective agents influence the freezing behavior of the solution principally by changing the phase diagram of the solution.

7. The temperature history is typically described in terms of a cooling rate. During rapid cooling, there is insufficient time for water to leave the cell in response to the increase in extracellular solution concentration resulting from the removal of water in the form of ice (due to freezing). Undercooling of the cell relative to the extracellular

solution results in intracellular ice formation, a lethal event, while slow cooling can result in excessive dehydration of the cell which is also damaging to the cell. The relative water content of a cell during freezing is a function of the cell type, with each cell type exhibiting its own unique biophysical characteristics, and the solution composition in which the cell is suspended.

8. The graph below shows the post-thaw survival and the optimal cooling rate for red blood cells cryopreserved in glycerol-containing cryopreservation solutions. As shown in this graph, both the optimal survival and the cooling rate at which this survival is observed vary with the composition of the solution.



9. McGrath and Diller, Low Temperature Biotechnology, Emerging Applications and Engineering Contributions (BED, 10, 380 (1988), a copy of the Contents is attached hereto), summarizing the work of many different studies, relate that cells which can be successfully cryopreserved exhibit a wide range of permeabilities to water (several orders of magnitude difference). For example, for the same